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23. (New) The fusion polypeptide of claim 21, wherein the α1,3 galactosyltransferase is porcine.

24. (New) The fusion polypeptide of claim 21, wherein said first polypeptide includes an extracellular portion of a P-selectin glycoprotein ligand-1.

25. (New) The fusion polypeptide of claim 21, wherein the second polypeptide comprises a region of a heavy chain immunoglobulin polypeptide.

26. (New) The fusion polypeptide of claim 25, wherein said second polypeptide comprises an Fc region of an immunoglobulin heavy chain.

27. (New) A method for treating a hyperacute rejection reaction in a subject in need thereof, the method comprising withdrawal of plasma from the subject, bringing said plasma in contact with the fusion polypeptide of claim 21 to couple anti-pig antibodies thereto and thereafter reinfusing said plasma to said subject.

28. (New) The fusion protein of claim 21, wherein the first polypeptide comprises more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1 polypeptide.

REMARKS

Upon entry of the foregoing amendments, claims 21-28 are under consideration.

New claim 21 is supported in the specification, *e.g.* page 4, lines 11-22 page 4, line 34 to page 5, line 5, page 5, lines 26-34, and originally filed claims 1, 4 and 9. Specifically, support for the fusion polypeptide as a dimer can be found in the specification, *e.g.*, page 4, lines 29-30, page 11, lines 23-25, and Figure 1 (substitute sheet). New claim 22 is supported in the specification, *e.g.* page 4, lines 9-10, and by original claim 1. New claim 23 is supported in the specification, *e.g.* page 4, lines 20-22, and original claim 4. New claim 24 is supported in the specification, *e.g.* page 5, lines 6-9 and by original claim 8. New claim 25 is supported in the specification, *e.g.* page 5, lines 26-34, and by original claim 9. New claim 26 is supported in the specification, *e.g.* page 5, lines 36-38, and by original claim 10. New claim 27 is supported in the specification, *e.g.* page 7, lines 1-13, and by original claim 20. Support for new claim 28 appears in the specification, *e.g.* page 4, line 27-29. Thus, these amendments add no new matter.

The §112, Second Paragraph Rejections

The Examiner has rejected claims 1-6, 8, 10-14 and 20 under 35 U.S.C. §112, second paragraph as being indefinite, alleging that the phrase "immunoglobulin properties" has not been

give a reasonably precise definition in the specification. Applicants have cancelled claims 2, 5, 6 and 12-14 and have amended claim 1 such that the phrase "immunoglobulin properties" has been deleted. This rejection should be withdrawn.

The §103(a) Rejections

A. Tsuji/Sako/'131

The Examiner has rejected claims 1-6, 8, 10-11, over Tsuji *et al.*, Chem. Pharm. Bull. **38(3)**:765-768, 1990 ("Tsuji") and US Patent No. 5,434,131 ("the '131 patent") in view of Sako *et al.* Cell **75**:1179-1186, 1993 ("Sako"). Applicants have cancelled claims 2, 5, and 6 and, in view of the claim amendments, traverse the Examiner's rejection of claims 1, 3-4, 8, and 10-11. The Examiner states that "Applicant offers insufficient evidence that the instant product specifically binds more anti-Galα1,3 Gal antibodies than the fusion protein of the *combined* prior art nor has Applicant provided sufficient evidence that said binding would be unexpected" (emphasis in original).

The present invention discloses in one embodiment a dimeric fusion protein consisting of a portion of a mucin, *e.g.* PSGL-1, and a region of an immunoglobulin polypeptide, that contains Galα1,3 Gal epitopes, for the purpose of removing anti-Galα1,3Gal antibodies. Applicants have amended claims 1, 3-4, 8, and 10-11 to clarify the patentable distinctions between the instant invention and the above-cited references. As amended, claim 1 recites a dimerized fusion polypeptide comprising a) a first polypeptide which comprises at least a region of a P-selectin glycoprotein ligand-1 and is glycosylated by an α1,3 galactosyltransferase and b) a second polypeptide comprising at least a region of an immunoglobulin polypeptide.

As a preliminary matter, there is no suggestion to combine Tsuji, '131 and Sako to produce the claimed invention. Tsuji merely refers to an antibody (lacking a mucin domain and other recited features), the '131 patent refers to a fusion protein lacking either mucin domains or Galα1,3Gal epitopes, and Sako refers to an endogenous protein lacking Galα1,3Gal epitopes. Furthermore, even if combined (which applicants do not believe is proper) that combination does not teach or suggest the claimed dimerized fusion protein.

Tsuji is critically deficient. Tsuji describes the isolation of a natural antibody from human sera which binds recombinant tissue plasminogen activator (tPA) and recombinant

erythropoietin (EPO) produced in C127 cells via α1-3-linked galactose residues on their sugar side chains. The identified antibody was capable of binding to natural tPA from blood vessels and placenta, but was incapable of recognizing recombinant tPA, EPO or protein C expressed in a Chinese hamster ovary (CHO) cell line.

Tsuji does not teach or suggest the creation of a fusion protein containing amino acid residues bearing both Galα1,3 Gal sites and a mucin-containing domain, as required by the amended claims. Tsuji lacks a mucin-containing domain, or any motivation to use one, as required by the present invention. Tsuji also does not teach or suggest glycosylation of the protein by an α1,3-glycostransferase. The consequence of this is that the Tsuji protein, unlike the claimed protein, lacks α 1,3 linkages required in the claimed protein (and methods of using it). This is made clear in Tsuji, by the failure to observe binding of the natural antibody to the Tsuji CHO-produced recombinant proteins -- cells which lack α1,3-galactosyltransferase.

Sako does not cure these deficiencies. Sako describe the cloning and partial characterization of a P-selectin glycoprotein ligand (PSGL-1) from a human leukemia cell line cDNA library expressed in COS cells. PSGL-1 is glycosylated with Lewis^x or sialylLewis^x moieties but lacks Galα1,3 Gal epitopes. Nor does Sako suggest the use of PSGL-1 to remove Galα1,3 Gal-binding antibodies. Further, Sako does not suggest that PSGL-1 should be modified to include Galα1,3 Gal sites. Therefore, the combination of Tsuji and Sako cannot lead the ordinarily skilled artisan to the claimed protein or methods.

Further, the combination of Tsuji and Sako with the '131 patent does not render obvious the claimed invention. The '131 patent teaches the use of a fusion protein consisting of the extracellular domain of CTLA4 and the hinge, CH2 and CH3 regions of human immunoglobulinCγ1. The '131 patent lacks a mucin domain and lacks Galα1,3 Gal epitopes. Nor does the '131 patent suggest including a mucin domain or Galα1,3Gal epitopes in a dimerized recombinant fusion protein, as is required in the amended claims.

Even assuming that the combination of Tsuji, Sako and the '131 patent could be made, that combination would not lead the ordinarily skilled artisan to the claimed protein -- a dimerized recombinant fusion protein with a mucin domain, an immunoglobulin domain and multiple Galα1,3 Gal epitopes generated by the activity of a exogenously expressed α1,3

galactosyltransferase. Therefore, Applicants assert that the rejection of claims 1, 3-4, 8 and 10-11 should be withdrawn.

B. Tsuji/Sako/'131/Goding

The Examiner has also rejected claims 12 and 14 over Tsuji in view of the '131 patent and Sako, in further view of Goding, Monoclonal antibodies: Principles and Practice, Academic Press, 1983 ("Goding"). Claims 12 and 14 have been cancelled. Therefore this rejection is moot.

C. Tsuji/Sako/'131/Kozlowski

The Examiner has also rejected claim 20 over Tsuji, the '131 patent and Sako in view of Kozlowski *et al.*, *Transplant Proc.* **29(1-2)**: 961, 1997 ("Kozlowski"). Claim 20 has been amended to be consistent with the language of claim 1 as amended. As amended, claim 20 is drawn to a method for treating a hyperacute rejection reaction in a subject in need thereof, wherein the method comprises withdrawal of plasma from the subject, bringing said plasma in contact with the fusion polypeptide of claim 1 to couple anti-pig antibodies thereto and thereafter reinfusing said plasma to the subject.

Kozlowski teaches a method of removing Galα1,3 Gal reactive natural antibodies from primate circulation by either (1) perfusion of whole blood through a pig liver; (2) perfusion of whole blood through an affinity column bearing Galα1,3Gal residues; or (3) perfusion of plasma separated from cellular components by apheresis using an affinity column. Methods (1) and (3) of Kozlowski are clearly not relevant here. Regarding method (2) of Kozlowski (the affinity column of the second method of Kozlowski), unlike the claimed protein of the instant application, that method does not involve use of the fusion protein of claim 1 (or any protein for that matter), and specifically lacks a mucin polypeptide domain. There is no teaching or suggestion in Kozlowski (or the other references in the combination) to modify the affinity column of Kozlowski to contain a mucin domain. Likewise, there is no motivation to modify the affinity column of Kozlowski to contain an immunoglobulin polypeptide. For this reason, the combination of Tsuji and the '131 patent and Sako in view of Kozlowski cannot lead to the claimed invention of claim 20. The rejection should be withdrawn.

Version With Markings to Show Changes Made

Claims 1-6, 8, 10-14 and 20 were cancelled.

Claims 21-28 are new.

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CONCLUSION

Applicants believe that the claims, as amended, are in condition for allowance. If the Examiner has any questions, the Examiner is invited to contact the undersigned by telephone.

Respectfully submitted,

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